Absence of Pathognomonic or Inflammatory Patterns in Cardiac Biopsies From Patients With Brugada Syndrome

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Background—Brugada syndrome (BrS) is characterized by the presence of coved ST-segment elevations in the right precordial leads (so-called type I ECG) and additional clinical features. Caused by cardiac ion channel gene mutations, BrS may be associated with ventricular and atrial conduction disturbances as well as ventricular fibrillation. Recent studies have discussed whether BrS is merely a primary electric disorder or whether inflammatory or other histopathologic abnormalities in the right ventricle (RV) underlie the ECG phenotype.

Methods and Results—We retrospectively analyzed BrS biopsy samples from 21 unrelated patients for histopathologic abnormalities (hypertrophy, fibrosis, inflammation, fatty tissue) together with the patients’ clinical, genetic, and imaging data. Eleven patients (52%) had normal RV imaging (by angiography, echocardiography, or cardiac MRI). Results of myocardial biopsies were normal in 3 patients (14%) and revealed mostly moderate abnormalities in the others. Four patients (19%) had predominant fatty tissue in the RV myocardium. Using immunohistochemistry and conventional tissue staining, we could not detect inflammatory tissue changes, an observation compatible with the clinical absence of signs for myocarditis.

Conclusions—Imaging and histopathologic evaluation may detect moderate but uncharacteristic cardiac abnormalities in patients with BrS. None of the patients had arrhythmogenic RV cardiomyopathy or overt myocarditis. Only in a small subset did predominant histopathologic abnormalities in the biopsy samples of the RV outflow tract occur that could provide a link to the ECG phenotype. A variety of mechanisms, including genetic and structural RV alterations, may underlie the Brugada ECG phenotype. (Circ Arrhythmia Electrophysiol. 2009;2:16-23.)

Key Words: Brugada syndrome | ARVC | biopsy | SCN5A | myocarditis

An ECG published in 1953 by Osher and Wolff3 was probably the first ECG pattern resembling that found in Brugada syndrome (BrS).2,3 More than 3 decades later, investigation of 2 series of patients with this ECG pattern led to further recognition of BrS as a clinical entity.3 In the first series reported by the Brugada brothers,3,4 the characteristic ECG pattern of ST-segment elevation, conduction delay, and preterminally negative T-waves (so-called coved-type or BrS type I ECG) in the right precordial leads (V1–V2(3)) was associated with sudden cardiac death in the presence of a “structurally normal heart.”

Clinical Perspective see p 23

In 1998, BrS was classified as a genetic disorder owing to the first identification of heterozygous mutations in the gene encoding the α-subunit of the cardiac sodium channel (SCN5A).5 However, only one third of patients with BrS, mainly those with a positive family history, have a mutant, mostly nonfunctional SCN5A protein.6 Since 1998, further genetic heterogeneity has been described.7–10 Similar to congenital long-QT syndrome and other cardiac ion channel disorders, BrS was initially proposed as a primary “electric heart disease”11 in anticipation that cellular, but not gross macroscopic, structural changes would be associated with the syndrome. However, this proposal was challenged when evidence was found for histological changes in the right ventricular (RV) myocardium (RV cardiomyopathy) of patients with type I ECG.12 In some cases, histopathologic criteria could be used to diagnose arrhythmogenic RV cardiomyopathy (ARVC) after autopsy.13 In addition, in victims of sudden cardiac death due to BrS, histopathologic examination showed fibro-fatty replacement in the RV wall and...
fibrosis in the conduction system.\textsuperscript{13-15} Beyond single case reports, only one study has so far addressed morphological changes shown in biopsies of the RV (and left ventricle [LV]) of patients with BrS.\textsuperscript{16} A prevalent or localized RV myocar-
dial changes shown in biopsies of the RV (and left ventricle [LV]) has been reported as a key finding in 14 of 18 patients with BrS, as well as a significant increase of apoptotic myocytes in RV and LV.\textsuperscript{16} These histopathologic cardiac findings have raised the possibility of other, perhaps nonge-
netic, causes with clinical features similar to those of BrS.

To further address other causes of the diagnostic type I ECG phenotype in BrS, we retrospectively evaluated RV biopsy samples and genetic and clinical data from 21 unre-
related, consecutively identified patients with typical BrS.

**Methods**

**Patient Population and Clinical Assessment**

Twenty-one consecutive patients with the ECG diagnosis of BrS (type I ECG) were studied (Table 1). The criteria for the diagnosis of type I ECG were applied according to the latest consensus expert report.\textsuperscript{2} This characteristic ECG pattern was observed either under baseline conditions or when it appeared after ajmaline challenge. All patients were investigated for additional features of BrS, including syncope, resuscitation, nocturnal agonal respiration, polymorphic ventricular tachycardia or ventricular fibrillation during programmed ventricular stimulation, a positive family history (sudden cardiac death at <45 years or the presence of BrS), or the presence of an indicative SCN5A mutation.\textsuperscript{4} We also investigated by routine cardiac imaging techniques (transthoracic echocardiography, 1.5-T MRI with gadolinium contrast, laevo- and dextrocardiography; Table 2) the presence of gross structural heart or intrathoracic diseases (eg, extracardiac RV outflow tract [RVOT] compression) that are known to mimic the BrS ECG phenotype.\textsuperscript{5} In all patients, programmed ventricular stimulation was performed.\textsuperscript{17} To exclude hidden or cardio-
myopathic RV changes, we took endomyocardial biopsy samples from

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<th>ICD</th>
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PVS indicates programmed ventricular stimulation; VT, ventricular tachycardia; VF, ventricular fibrillation; pVT, polymorphic VT; NS, nonsustained; m, male; f, female; ICD +, implantable cardioverter-defibrillator implanted; ICD −, no implantable cardioverter-defibrillator implantation.

Histopathology

Myocardial specimens were fixed in buffered formalin and embed-
ded in paraffin. Five-micrometer thick sections were stained with
hematoxylin-eosin and Miller’s elastic Van Gieson to evaluate
fibrotic and fatty changes. All BrS biopsy samples were analyzed
and quantified independently by up to 3 cardiac pathologists who
were blinded to diagnosis. A semiquantitative score (0, no changes;
1, moderate changes; 2, predominant changes) was used to describe
the degree of myocellular hypertrophy, fibrosis, inflammation, and
presence of fatty tissue. Because the occurrence of pure fatty
infiltrates of the RV may be a normal finding, we considered changes
to be moderate (score 1) with fatty tissue of >10% after morphometric analyses. Burke et al\textsuperscript{18} reported a similar value from RV myocardial biopsy samples of normal-weighted controls after morphometric analyses.
Although up to 15% fatty replacement is reported to be abnormal in the RVOT and posterior wall, fatty replacement may be considered normal in the anterior and apical parts of the RV.\textsuperscript{19} The presence of >5% of fibrous tissue was considered moderate (score 1).

For the histopathologic diagnosis of ARVC, we referred to a publication by Angelini et al.,\textsuperscript{20} who reported a relative amount of >80% of residual myocytes as normal (score 0) after histomorphometric investigations of endomycardial biopsies from 29 patients with ARVC, 30 with dilated cardiomyopathy, and 30 controls. Together with a relative amount of >40% of fibrous tissue and >3% of fatty tissue, sensitivity of these parameters was 67% and specificity 92% for the diagnosis of ARVC.\textsuperscript{20} In all patients with ARVC based on current diagnostic criteria\textsuperscript{21} and in consideration of histomorphometric criteria,\textsuperscript{20} the presence of ARVC was positive or suggestive for ARVC.

For detection of myocellular inflammation, we performed lymphocyte immunohistochemistry for the leukocyte-common antigen (CD45) by using a monoclonal mouse antibody (clone PD7/26; DAKO, Hamburg, Germany) as primary antibody and universal streptavidin-biotin-technique with alkaline phosphatase (all from DAKO). The average number of detected lymphocytes in representative views was analyzed in a high-power field (HPF) image. Results were compared in an unblinded manner with control samples (n = 12) that were obtained during the first myocardial biopsy after heart transplantation (International Society for Heart and Lung Transplantation).

The relative amount of fatty tissue was analyzed semiquantitatively and morphometrically by using CellA analysis software (Olympus Soft Imaging Solutions). The extent of fatty tissue was measured as an area and calculated as a percentage of the total area of the biopsy. Statistic features of Microsoft Excel 2002 were used to analyze basic statistics. Results were expressed as mean ± SD.

### Results

#### Clinical Data

Clinical characteristics of the 21 patients with BrS (16 males, 5 females; mean age, 43.9 ± 17.1 years) are shown in Table 1. Seven of the 21 patients with BrS (33%) presented with the BrS type I ECG pattern at baseline (ECGs not shown). In 11 patients (52%), the BrS type I ECG became apparent only after ajmaline challenge (ECGs not shown). In all patients, \( e \) waves suggestive of ARVC were not seen. Genetic analysis revealed a heterozygous SCN5A mutation in 7 patients (33%; 5 males, 2 females).\textsuperscript{6} None of the 21 patients had a history of myocarditis, nor did they have clinical signs, ECG alterations, or symptoms indicative of myocarditis.

The family history for sudden cardiac death was positive in only 6 patients with BrS; thus, in most cases, the disease was sporadic. In none of the probands was the family history positive or suggestive for ARVC.

During programmed ventricular stimulation, a sustained ventricular arrhythmia was inducible in 13 patients (62%; ventricular fibrillation, n = 11; ventricular tachycardia, n = 2); of these patients, only 4 presented with the BrS type I ECG at baseline and 2 had a heterozygous SCN5A mutation.

#### RV Imaging

Imaging findings of the 21 patients with BrS are summarized in Table 2. In most patients with BrS, imaging techniques revealed an almost normal RV function and structure. There were also no RVOT abnormalities. Late tissue enhancement in RV (and LV) during cardiac MRI was absent in all
investigated patients. On RV angiography (n = 20), RV function was mostly normal, although—as a nonspecific finding—trabecularization was found in the RV. Other more specific alterations in the RV, such as microaneurysms, bulging, or localized sacculations (reported from patients with ARVC or previously in BrS), were not found.

**Histopathologic Findings and Immunohistochemistry**

Histopathologic characteristics and biopsy sampling location in the 21 patients with BrS are shown in Table 3. The total number of samples was 3 to 5 per patient. Typically, the biopsy specimens were taken from the RVOT/mid-RV in 76% (16 of 21) of patients, in the septum in 86% (18 of 21), and in the RV apex in 57% (12 of 21). Biopsy samples of the LV were not taken.

Overall, there were no signs of active myocardial inflammation in any of the biopsy samples as addressed by hematoxylin-eosin staining and immunohistochemistry (Figure 1). Three patients had completely normal cardiac histopathologic findings (Table 3). Major findings in patients with histopathologic changes were moderate (score 1) myocardial hypertrophy (in 11 of 21 [52%]), moderate fibrosis (score 1, in 5 of 21 [24%]), and fatty replacement of the myocardium (in 10 of 21 [47.6%]). In 4 of the 10 patients, the extent of fatty tissue was predominant (score 2; Figure 2) and sample origins were septum (n = 2), RVOT (n = 1), and RV apex (n = 1). For hypertrophy and fibrosis, no predominant occurrence (score 2) was seen. Histopathologic findings, in particular the presence of fatty tissue, in cardiac BrS samples were unrelated to the absence of an SCN5A gene mutation (Table 3).

In the control group of samples (first myocardial biopsy after heart transplantation without signs of cellular rejection/International Society for Heart and Lung Transplantation 0; n = 12), only moderate (score 1) histopathologic changes were seen: hypertrophy in 50%, fibrosis in 50%, and fatty tissue in 25%. Only 3 patients (25%) had no histological alterations in the myocardium.

In only 2 BrS samples (patients 13 and 18), moderate (score 1) fibrotic and fatty changes occurred together. In the 4 samples with a predominant fatty tissue (score 2), semiquantitative morphometric assessment showed a variable amount of fatty tissue (range, 10% to 74%) in contrast to that shown in control samples (range, 0.7% to 2.5%) and other BrS samples (score 1; range, 0.4% to 5.8%).

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<th>Fatty Replacement*</th>
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BrS (n = 21): SCN5A+: 33.3% RVOT/mid-RV (76%), septum (86%), apex (57%)
Normal (47.6%) Normal (76.2%) Normal (52.4%) Normal (100%) 1.5±1.2

Controls (n = 12): NP Septum (100%) Normal (50%) Normal (50%) Normal (75%) Normal (100%) (ISHLT 0) 1.8±1.2

Data are from control samples (summarized).
+ indicates present; −, absent; NP, not performed.
*0 indicates none; 1, moderate; 2, predominant (see Methods section).
†Semiquantitative number of lymphocytes detected under HPF after immunohistochemistry detection.

Table 3. Histopathologic and Immunohistochemical Findings in Cardiac Biopsy Samples of 21 Patients With BrS
Because of the obvious but variable presence of fatty tissue of the myocardium in 10 patients, their medical history was reevaluated for the presence of diagnostic criteria for ARVC. Six patients with a score of 1 did not meet ARVC criteria. The 4 patients from whom samples were taken that showed predominant fatty replacement (score 2) displayed the typical, diagnostic type I ECG at baseline (n/H110051) or after ajmaline challenge (n/H110053; not shown). The predominant occurrence of fat in the RV apex of patient 19 was considered nonspecific because it was noninfiltrative and merely localized at the margin. In summary, in the 4 patients with predominant fat occurrence, neither histopathologic nor clinical features compatible with ARVC were seen.

Unlike the authors of a recent study,16 we did not find any evidence for inflammatory infiltrates or localized myocarditis in our series that may mimic the Brugada-type ECG. After immunostaining for leukocyte-common antigen, HPF analysis of BrS samples showed an average of 1.5±1.2 lymphocytes per HPF, comparable with that of the control sample group (1.8±1.2 per HPF; n=12).

Discussion

BrS has recently become more recognized as an entity with a characteristic, but sometimes varying, ECG presentation2,22 and associated clinical features. Individuals with BrS are at higher risk for sudden cardiac death, and clinical overlap with other sudden death syndromes (eg, BrS and conduction disease or long-QT syndrome23; SCN5A mutation and dilated cardiomyopathy24) is known. Because inborn defects in cardiac ion channel genes have been identified,5,8–10 the BrS ECG phenotype was assumed to reflect a primary genetic and mainly electrophysiologic disturbance, which led to its classification as a primary electric heart disease. However, this classification has been questioned,25 because knowledge of causative genetic alterations is incomplete, and in many patients (in particular, sporadic cases), causal factors can be unidentified. In addition, acquired forms of BrS are known2,22 in which exogenous and nongenetic causes (conduction-slowing drugs, hyperthermia, RV/RVOT ischemia, or compression) may phenocopy primary BrS but produce reversible ECG alterations. This observation raises the possibility that in most patients, nongenetic conditions may underlie BrS.

Electric changes of cardiac ion currents in the RV can cause a Brugada ECG pattern under experimental conditions.26 In arterially perfused canine RV wedge preparations, intrinsic ionic current heterogeneities within the ventricular myocardium can be associated with ST-segment elevation similar to that observed in patients with BrS.26 Recently, in vivo studies indicated that, even in the presence of an electrophysiologic alteration (eg, caused by an ion channel [SCN5A] mutation), inhomogeneous, delayed conduction in the RV or the RVOT can be identified by tissue Doppler echocardiography or during directed ex vivo electrophysiologic studies.27,28 Along these lines, cardiac tissue from patients with BrS or mice models showed RV myocardial fibrosis, structural abnormalities, and conduction delay and...
supported evidence for pathogenetic mechanisms beyond merely electrophysiologic alterations, even in settings characterized by a reduced cardiac sodium current.27–31

Frustaci et al16 recently used a different approach to systematically investigate cardiac biopptic samples and clinical data from 18 patients with BrS. Microaneurysms were detected in the RV and sometimes in the LV (7 of 18 patients) by angiography, but not by transthoracic echocardiography. In the present series of 21 patients with BrS, we could not see these alterations retrospectively during RV angiography or cardiac MRI (Table 2) nor have we observed these alterations in other patients with BrS beyond the scope of the present investigation. In contrast to the study population of Frustaci et al,16 in which no imaging abnormalities were reported beyond the occurrence of microaneurysms, in our patients, we found slight RV alterations (hypokinetic RV in 6 patients, RV trabecularization in 4 patients), but no microaneurysms. However, our imaging findings can be considered a normal result of temporarily inhomogeneous contractions in apical and anteroseptal RV areas. Because the RVOT has been reported as the area of electrophysiologic and structural abnormalities,27,28 other cardiac imaging studies have focused in particular on abnormalities in this anatomic area. In one cardiac MRI study of 20 patients with BrS, the RVOT tract area was significantly enlarged but without overt structural abnormalities when compared with controls.32 Similarly, an electron beam computed tomography study by Takagi et al33 noted morphological abnormalities in the RVOT (17 of 26 patients) or in the inferior RV wall (n=4) in most patients. Although these sites corresponded to the origin of premature ventricular contractions, it remained unknown whether these contraction abnormalities could also be recorded in the absence of premature ventricular contractions.33 In contrast, Tukkie et al37 found no RV wall abnormalities, but did note an onset delay of RV activation and contraction during tissue Doppler imaging. In transthoracic echocardiography, only a small portion of patients (19%, n=3) showed a mild dilatation of the RV in our series. Thus, subtle, or overt but mild, structural changes in the RVOT/RV can be detected in BrS and may represent localized, arrhythmogenic substrates. In addition, cardiac imaging abnormalities were not related to the genetic status of the patient (SCN5A+ versus SCN5A−) and were not compatible with the presence of RV cardiomyopathy (such as ARVC).

**Histopathologic Changes**

In the present study, in most myocardial biopsy samples (76% from the RVOT/mid-RV, 86% septal, 57% apical), we saw only moderate changes that were unlikely to represent the arrhythmogenic substrate or to correlate with the particular ECG phenotype. In 4 patients, we saw extensive fatty replacement (Figure 2, Table 3), although this can be a nonspecific finding, particularly at the RV apex.34

Although the evidence is not conclusive, we consider most moderate changes to be related to the BrS phenotype. Careful clinical investigations (various imaging techniques, surface ECGs) in these cases were not sufficient to diagnose ARVC with the current diagnostic scoring system.21 In addition to localized (eg, triangle of dysplasia) or diffuse structural and contractile RV dysfunction, histopathologic changes are a major criterion. These changes are characterized by either an infiltrative pattern (continuous fibrous replacement of the RV myocardium extending from the subepicardial to a transmural layer) or a cardiomyopathic pattern.35,36 The latter resembles more extensive disease and shows a prominent fibro-fatty replacement and degenerative changes, myofibril loss, RV wall thinning, and aneurysms, as well as microscopic features of focal myocarditis.35,36 The diagnosis of ARVC, however, is not based on histopathologic findings alone,21 because other conditions, eg, adipsitas cordis, may lead to a similar histopathologic appearance. However, residual myocytes of <45% (normal >80%), fibrotic tissue of >40%, and fatty replacement of >3% in endomyocardial biopsy specimens have been reported to have a sensitivity of 67% and specificity of 92% for ARVC.20 In none of the presented samples have we seen such a pattern or a predominant fibrotic tissue replacement. We conclude that different degrees of fibrous and/or fatty replacement can also occur in a subset of patients with BrS that were diagnosed for BrS according to current diagnostic criteria.2,22 Thus, fibrous and/or fatty replacement of the RV myocardium may be a less specific finding than originally thought. Even in the control sample of first biopsies after heart transplantation (International Society for Heart and Lung Transplantation 0), 25% showed adipose tissue to a minor extent and 50% showed fibrotic changes (Table 3).

We found no histopathologic changes in only a few patients with BrS (n=3; 14.3%). The presence of an SCN5A mutation as an indicator for ion channel disease and BrS was associated with moderate histopathologic changes, as noted in earlier studies.28,37 Along with SCN5A knock-out mouse models29–31 and reports from other ungenotyped patients,12 evidence exists for at least fibrotic changes in the RV myocardium of patients with BrS. In our study, we found fibrotic changes in the RVOT in 5 patients (24%). In a BrS patient’s explanted heart that was previously considered to have no structural abnormality, the RVOT/RV showed fibrosis and endocardial fatty infiltration (typical for ARVC) that was focally interspersed with hypertrophic cardiomyocytes, together with changes in electric propagation.29 In our series, fibrotic or fatty tissue was seen in 62% (n=13) of samples. From our experience, moderate fibrous or fatty replacement is more frequent in cardiac BrS samples than was recently noted by Frustaci et al.16 Unlike these investigators, we did not find differences in histopathologic alterations in the presence or absence of an SCN5A mutation, which we considered as an independent observation. On the other hand, cardiac sodium channel disease can be associated with various detectable tissue changes in the RV myocardium that are likely to be secondary changes because they can be seen in other subsets of patients with BrS.

To date, only one study has comprehensively investigated biopsy specimens from 18 patients with BrS.16 Most samples (n=14, 78%) showed localized RV myocarditis with lymphocytic infiltrates and adjacent necrosis, including 4 cases of successful viral genome detection.16 None of the cases had an SCN5A gene mutation. Microaneurysms were detected during angiography in the RV in 7 of 18 patients. In comparing the
 imaging data from our patients with the data in the study by Frustaci et al., it is obvious that the 2 study populations are different because microaneurysms, bulging or localized sacculations, and specific findings indicative of ARVC or previously reported in BrS were not found in the present study. Regarding lymphocytic infiltrations and the presence of myocarditis, a key finding of the previous study, our patient population did not show either clinical or imaging signs for myocarditis or lymphocytic infiltrates during routine staining or after immunohistochemistry. Moreover, HPF analysis showed a similar presence of lymphocytes when compared with the given control group. Other signs of cardiomyopathic changes (eg, diffuse vacuolization and cytoplasm degeneration), were not seen, again indicating that the patient population of the present study does not have an inflammatory background and acquired cause for BrS. Because of the retrospective nature of the present study, and because of the normal presence of lymphocytes without infiltration, we did not perform polymerase chain reaction to detect viral genomes as surrogate markers for myocarditis, assays to detect myocellular apoptosis (eg, TUNEL assay), and assessment of ultrastructural changes to the inherited primary ion channel dysfunction. The notion that concealed but extensive and localized fibrous and/or fatty replacement can be observed in BrS may further strengthen the hypothesis that replacement of myocytes is not a unique feature of ARVC and may be detectable in BrS (eg, in the setting of a common cardiomyopathic pathway). We consider these predominant fatty changes as a potentially arrhythmogenic substrate that may lead to a localized conduction delay. On the other hand, from clinical observations, ARVC and BrS are 2 distinguishable conditions that have marked differences with respect to the genes involved, clinical and ECG presentation, and autonomic modulation. So far only a single-genetic locus (ARVC-5) on chromosome 3p25 has been identified that may harbor a gene for both entities, because the 2 genetic candidate loci overlap. The gene (TMEM43) has recently been identified and may shed further light on the pathogenesis and potential clinical overlap. The proposed novel classification of cardiomyopathies based on molecular pathways (eg, desmosomal versus ion channel disease) will further promote discussion on the classification of congenital and acquired cardiomyopathies.

Limitations
Because of the detection of normal lymphocyte values in this retrospective study, we did not perform reverse transcriptase polymerase chain reaction to detect viral genomes as surrogate markers for myocarditis, assays to detect myocellular apoptosis (eg, TUNEL assay), and assessment of ultrastructural changes by electron beam microscopy. Control samples of cardiac transplant biopsies were analyzed in an unblinded fashion.

Conclusion
From our observations, myocardial biopsy samples from 21 patients with BrS can be used to detect minor histopathologic changes but do not have a pathognomonic pattern or lymphocytic infiltration when myocarditis is absent. The findings from RV imaging techniques were normal in most patients. The mostly moderate histopathologic alterations were independent from the genetic background of BrS and were not sufficient to explain the ECG phenotype or severity of the clinical course. These changes may be considered as nonspecific and further confirm the heterogeneous background and complexity of this syndrome.

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References


**CLINICAL PERSPECTIVE**

Brugada syndrome is a cardiac ion channel disorder that puts individuals at risk for sudden cardiac death. Currently, genetic defects account for only a subset of patients; therefore, other disease mechanisms must be taken into account. In the present study, a large set of myocardial samples excluded an inflammatory cause. In addition, no overlap with arrhythmogenic right ventricular cardiomyopathy was observed. Taken together, these results indicate that routine histological investigations in patients with Brugada syndrome are not useful in ascertaining a clinical diagnosis.
Absence of Pathognomonic or Inflammatory Patterns in Cardiac Biopsies From Patients With Brugada Syndrome
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